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Cinnamon Polyphenol Extract Affects Immune Responses by Regulating Anti- and Proinflammatory and Glucose Transporter Gene Expression in Mouse Macrophages[1–3]

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Abstract

Tristetraprolin (TTP/zinc finger protein 36) family proteins have antiinflammatory effects by destabilizing proinflammatory mRNA. TTP expression is reduced in fats of obese people with metabolic syndrome and brains of suicide victims and is induced by insulin and cinnamon polyphenol extract (CPE) in adipocytes, by lipopolysaccharide (LPS) in macrophages, and by green tea polyphenol extract in rats. CPE was reported to improve immune function against microorganisms, but the mechanism is unknown. This study tested the hypothesis that CPE regulates immune function involving genes encoding TTP, proinflammatory cytokines, and glucose transporter (GLUT) families and compared the effects of CPE to those of insulin and LPS in mouse RAW264.7 macrophages. CPE increased TTP mRNA and protein levels, but its effects were less than LPS. CPE (100 mg/L, 0.5–4 h) increased TTP and tumor necrosis factor (TNF) mRNA levels by up to 2- and 6-fold that of the control, respectively, and the base level of TTP was 6-fold that of TNF. LPS (0.1 mg/L, 4 h) increased TTP, TNF, granulocyte-macrophage colony-stimulating factor, cyclooxgenase-2, and interleukin 6 mRNA levels by 39–1868 fold. CPE and LPS increased GLUT1 expression (the major GLUT form in macrophages) to 3- and 2-fold that of the control, respectively. Insulin (100 nmol/L, 0.5–4 h) did not exhibit major effects on the expression of these genes. CPE increased TTP expression more rapidly than those of proinflammatory cytokines and the net increases of TTP mRNA levels were larger than those of proinflammatory cytokines. These results suggest that CPE can affect immune responses by regulating anti- and proinflammatory and GLUT gene expression. J. Nutr. 138: 833–840, 2008.

Introduction

Tristetraprolin [TTP[4]/zinc finger protein 36 (ZFP36)/tetradecanoyl phorbol acetate (TPA)-induced sequence 11 (TIS11)/ G0/G1 switch gene 24 (G0S24)/nuclear protein 475 (NUP475)] family proteins regulate gene expression at the post-transcriptional level. TTP family proteins consist of 3 well-known members in mammals [ZFP36 or TTP, ZFP36L (ZFP36-like)-1 or TIS11B, and ZFP36L2 or TIS11D] and the 4th member in mouse and rat but not in humans (ZFP36L3) (1,2). TTP is the product of the immediate-early response gene *Zfp36* in the mouse and *ZFP36* in humans (3–5). It binds to AU-rich elements in some mRNA and destabilizes those transcripts encoding proteins, such as tumor necrosis factor-α(TNFα) (6–9), granulocyte-macrophage colony-stimulating factor/colony-stimulating factor 2 (GM-CSF/CSF2) (10,11), and cyclooxygenase 2/prostaglandin-endoperoxide synthase 2 (COX2/PTGS2) (12). The mRNA encoding TNFα and GM-CSF are stabilized in TTP knockout mice and in cells derived from them (8,10). Excessive levels of these cytokines in TTP knockout mice results in a severe systemic inflammatory syndrome, including arthritis, autoimmunity, and myeloid hyperplasia (13,14). Up-regulation of TTP reduces inflammatory responses in macrophages (15). These lines of evidence support the conclusion that TTP is an antiinflammatory protein.

Agents that induce TTP gene expression may have potential therapeutic value for the prevention and/or treatment of inflammation-related diseases. TTP mRNA and protein levels are induced by a number of agents, including growth factors (3,4), cytokines [TNFα, GM-CSF, and interferon-γ (IFNγ)] (4,5,8,15), zinc (16), and plant nutritional products (cinnamon and green tea)

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[3] Supplemental Tables 1 and 2 are available with the online posting of this paper at jn.nutrition.org.
[4] Abbreviations used: CPE, cinnamon polyphenol extract; COX2 (PTGS2), cyclooxgenase-2 (prostaglandin-endoperoxide synthase 2); C_T, cycle of threshold; DMSO, dimethylsulfoxide; GLUT, glucose transporter; GM-CSF (CSF2), granulocyte-macrophage colony-stimulating factor (colony-stimulating factor 2); GSK3B, glycogen synthase kinase-3 β; HuR (ELAVL1), Hu antigen R (embryonic lethal, abnormal vision-like 1); IFNγ, interferon-γ; IL, interleukin; INSR, insulin receptor; LPS, lipopolysaccharide; RPL32, ribosomal protein L32; TNFα, tumor necrosis factor-α; TTP, tristetraprolin; VEGF, vascular endothelial growth factor; ZFP36, zinc finger protein 36; ZFP36L, ZFP36-like.
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(17,18). However, TTP gene expression is also induced by tumor promoters (3,5), bacterial endotoxin lipopolysaccharide (LPS) (8,19), and viral infection (20). The fact that most of these agents also increase the expression levels of proinflammatory cytokines such as TNFα in the same cells and/or tissues (8) may limit the therapeutic potential of these agents. Therefore, it is important to search for other agents with the potential to favor antiinflammatory and reduce proinflammatory gene expression.

Common spices (cinnamon, cloves, turmeric, and bay leaves) display insulin-like activity in vitro (21). Polyphenolic polymers from an aqueous extract of commercial cinnamon increase glucose metabolism in an epididymal fat cell assay (22). Cinnamon polyphenol extract (CPE) has other potential benefits for human health. For example, CPE was reported to exhibit activities against microorganisms (23), suggesting a potential role of CPE in regulating immune function; but the mechanism is unknown. Because CPE induces antiinflammatory TTP gene expression in mouse 3T3-L1 adipocytes (17), TTP is potentially involved in CPE-mediated immune regulation.

Mouse RAW264.7 macrophages are widely used as a cell model for inflammation research. TTP gene expression has been investigated extensively using this cell line (19,24). TTP protein was rapidly induced by LPS and accumulated in the cytosol of RAW264.7 cells (19). However, whether LPS also regulates the expression of the 3 TTP homologs in RAW264.7 macrophages is not known. In addition, glucose is the dominant metabolic substrate critically important for the host response to injury and infection (25,26). Glucose uptake is facilitated by glucose transporter (GLUT) family proteins in mammalian cells and GLUT1 is the major form in macrophages (27). We therefore utilized this model to evaluate the antiinflammation properties of CPE.

The objective of this study was to test the hypothesis that CPE regulates immune function involving genes encoding TTP, proinflammatory cytokines, and GLUT families, and compare the effects of CPE to those of insulin and LPS in RAW264.7 macrophages.

Materials and Methods

Materials and reagents. Mouse RAW264.7 macrophages were from American Type Culture Collection. DMEM was from Gibco BRL. Protein Assay Dye Reagent Concentrate and bovine serum albumin were from Bio-Rad. LPS and recombinant human insulin expressed in yeast were from Sigma. TRIzol reagent and SuperScript II RT were from Invitrogen. RNA 6000 Ladder was from Ambion. PCR primers and TaqMan probes were designed using Primer Express software (Applied Biosystems) and synthesized by Biosearch Technologies. Absolute QPCR mix was from ABgene House.

CPE. Water-soluble CPE was prepared as described previously (17,22). Briefly, ground cinnamon (*Cinnamomum burmannii*) was suspended in 0.1 mol/L acetic acid and autoclaved for 15 min at 15 psi. The supernatant was mixed with ethanol and refrigerated overnight followed by filtration through glass wool and Whatman no. 1 filter paper. The ethanol was removed by rotoevaporation and the remaining solution was freeze-dried. The dried CPE was reconstituted at 100 g/L in 100% dimethylsulfoxide (DMSO). The compositions of CPE mixture were analyzed by HPLC using a Symmetry Prep C_{18} column and were similar to those reported (22,23).

Cell culture and cell extracts. Mouse RAW264.7 macrophages were maintained as described (19) at 37°C in a humidified incubator with 5% CO_2 in DMEM containing 4500 mg/L (25 mmol/L) glucose supplemented with 10% (v:v) fetal bovine serum, 0.1 million units/L penicillin, 100 mg/L streptomycin, and 2 mmol/L L-glutamine. RAW cells were treated with 100 mg/L CPE (corresponding to 0.1% DMSO in the culture medium), 100 nmol/L insulin, or 0.1 mg/L LPS for 0, 15, 30, 45, 60, 90, 120, 180, and 240 min. The selection of the doses of CPE, insulin, and LPS was based on previous studies showing effective stimulation of TTP expression in adipocytes (17,28) and macrophages (19). Cell extracts were prepared as described (19). Protein concentrations in the 10,000-g supernatant were determined with modifications using the Protein Assay Dye Reagent Concentrate following NaOH treatment of the samples (7). Bovine serum albumin was used as the protein standard.

SDS-PAGE and immunoblotting. SDS-PAGE and immunoblotting were performed as described (6) using 10% SDS-PAGE and nitrocellulose membranes. The membranes were blocked with 5% nonfat dry milk and successively incubated in buffers containing the primary antibodies (1:1000 dilution) for 4 h and the secondary antibodies (1:10,000 dilution) for 1.5 h. Proteins on the immunoblots were detected using SuperSignal West Pico Chemiluminescent substrate (Pierce) followed by imaging with BioChemi Image Acquisition and Analysis System (UVP BioImaging Systems). The primary antibodies were anti-maltose binding protein-TTP and anti-MBP-ZFP36L1 sera raised against recombinant *Escherichia coli* maltose-binding protein fused to the full-length mouse TTP (19) or ZFP36L1/TIS11B (29). The secondary antibodies were affinity-purified goat anti-rabbit IgG (H+L) horseradish peroxidase conjugate with human IgG absorbed (Bio-Rad).

RNA extraction and real-time PCR analysis. Total RNA were isolated from mouse RAW264.7 cells treated with DMSO, CPE, insulin, or LPS using TRIzol reagent (Invitrogen). RNA concentrations and integrity were determined using RNA 6000 Nano Assay kit and the Bioanalyzer 2100 (Agilent Technologies) with RNA 6000 Ladder as the standards. The cDNA were synthesized from total RNA using SuperScript II RT as described (17). The gene names, GenBank accession numbers, amplicon sizes, and the sequences (5′ to 3′) of the forward primers, TaqMan probes (TET-BHQ1), and reverse primers, respectively, are described in **Supplemental Table 1**. TaqMan reaction mixtures and the thermal cycle conditions were identical to those described (17). PCR were performed in 96-well plates in an ABI Prism 7700 real-time PCR instrument (Applied Biosystems). The $\Delta\Delta$cycle of threshold (C_T) method of relative quantification was used to determine the fold-change in expression (17).

Statistical analyses. The real-time PCR data from the control, CPE, and insulin treatments with or without those from LPS treatment were analyzed separately by SigmaStat 3.1 software (Systat Software) using ANOVA or ANOVA on ranks. Multiple comparisons were performed with the Student-Newman-Keuls method. Differences were considered significant at $P < 0.05$.

Results

Relative expression levels of TTP family, GLUT family, and other selected genes in mouse RAW264.7 macrophages. Quantitative real-time PCR was used to evaluate the relative expression levels of TTP family genes (*Ttp/Zfp36*, *Zfp36l1*, *Zfp36l2*, *and Zfp36l3*) and other selected genes in mouse RAW264.7 cells (**Table 1**). ZFP36L2 mRNA was the most abundant molecule among the tested mRNA and its levels were >4-fold that of TTP in RAW264.7 cells. TTP mRNA levels (the 4th most abundant among those tested targets) were ~2- to 100-fold those of ZFP36L1 and ZFP36L3 (Table 1). Hu antigen R/embryonic lethal, abnormal vision-like 1 (HuR/ELAVL1) mRNA levels were the 2nd most abundant and were ~2-fold that of TTP. Vascular endothelial growth factor (VEGF) B mRNA levels were 19% less than those of TTP. TTP mRNA levels were ~6- to 10,000-fold those of TNF, GM-CSF, COX2, VEGFA, interleukin (IL) 6, and IFNγ in untreated RAW cells (Table 1).

We used PCR assays to evaluate GLUT family, insulin receptor (INSR), and glycogen synthase kinase 3-β (GSK3B)

TABLE 1 Relative levels of TTP family, GLUT family, and other selected mRNA in untreated mouse RAW264.7 macrophages[1,2]

mRNA	C_T	Expression ratio
		Fold of TTP or GLUT1
Ribosomal protein L32 (RPL32)	18.16 ± 0.12	Internal control
TTP (ZFP36/TIS11)	23.66 ± 0.29	1.00
ZFP36L1 (TIS11B)	24.81 ± 0.27	0.45 ± 0.10
ZFP36L2 (TIS11D)	21.59 ± 0.33	4.18 ± 0.89
ZFP36L3	30.13 ± 0.39	0.012 ± 0.003
TNF	26.24 ± 0.38	0.17 ± 0.03
GM-CSF (CSF2)	32.71 ± 0.51	0.002 ± 0.001
COX2 (PTGS2)	29.60 ± 0.38	0.02 ± 0.01
IL6	35.37 ± 0.80	0.0003 ± 0.0002
IFNγ	37.45 ± 1.06	0.0001 ± 0.0001
HuR (ELAVL1)	22.42 ± 0.12	2.18 ± 0.46
VEGFA	28.97 ± 0.30	0.03 ± 0.01
VEGFB	23.97 ± 0.21	0.81 ± 0.21
RPL32	18.19 ± 0.15	Internal control
GLUT1 (SLC2A1)	22.97 ± 0.37	1.00
GLUT2 (SLC2A2)	Undetectable	Undetectable
GLUT3 (SLC2A3)	25.96 ± 0.28	0.13 ± 0.03
GLUT4 (SLC2A4)	33.31 ± 0.28	0.0008 ± 0.0002
INSR	29.19 ± 0.21	0.014 ± 0.004
GSK3B	33.84 ± 0.45	0.0006 ± 0.0002

[1] Values are means ± SD, $n = 9$–22.
[2] RNA-derived cDNA (25 ng) was used for the quantitation of mRNA levels using 50 cycles of real-time PCR program. The relative ratios of mRNA levels were calculated using the $\Delta\Delta C_T$ method normalized with RPL32 C_T value as the internal control and TTP or GLUT1 C_T value as the calibrator.

mRNA levels in macrophages. GLUT1 mRNA was the 3rd most abundant among those tested, was the most abundant form of GLUT family in macrophages, and was 8- and 1300-fold those of GLUT3 and GLUT4 mRNA, respectively (Table 1). GLUT2 mRNA was undetectable by the PCR assay with 50 cycles (Table 1). INSR and GSK3B mRNA were detected in RAW macrophages, although their levels were much less than those of GLUT1 (Table 1).

CPE and LPS but not insulin increased antiinflammatory TTP mRNA and protein levels. Antiinflammatory TTP expression is induced by CPE and insulin in mouse 3T3-L1 adipocytes (17,28) and by LPS in RAW264.7 macrophages (19). We compared the effects of CPE, insulin, and LPS on TTP gene expression in macrophages, a well-established cell type for inflammatory response. RT-PCR analysis showed that CPE rapidly increased TTP mRNA levels in mouse RAW264.7 cells. TTP mRNA levels in cells treated with 100 mg/LCPE for 30–240 min were ~50–100% greater than those in the corresponding controls (**Table 2**). Insulin did not exhibit any major effect on TTP mRNA levels in RAW cells, except that its levels were slightly lower when cells were treated for 120 min (Table 2). LPS had a much larger effect on TTP gene expression in RAW cells. TTP mRNA levels in cells treated with 0.1 mg/L LPS for 30–240 min were 9- to 39-fold of the controls (Table 2).

Immunoblotting was used to investigate if CPE could induce TTP protein levels in RAW cells. TTP increased in cells treated with 100 mg/L CPE for 90–180 min (**Fig. 1**, lanes 6–8). Under similar blotting and exposure conditions, LPS increased TTP protein levels in RAW cells with much earlier and greater magnitude than CPE induction (Fig. 1, lanes 9–14). However, TTP protein levels were below detection in cells treated with insulin for the same length of time (data not shown).

CPE, LPS, and insulin effects on TTP homologs, VEGF, and HuR gene expression. TTP homologs (ZFP36L1, ZFP36L2, and ZFP36L3) are also capable of destabilizing proinflammatory cytokine mRNA molecules in transfected cells (1). We therefore analyzed the effects of CPE, LPS, and insulin on the regulation of these 3 TTP homologs in RAW cells. The effects of CPE on the expression of TTP homologs in RAW cells were generally small. CPE decreased ZFP36L1 mRNA levels by ~30% in cells treated for 30 and 240 min (Table 2). ZFP36L2 mRNA levels were decreased by 20% in cells treated for 30 min (Table 2). ZFP36L3 mRNA levels were also slightly decreased in RAW cells after 120- and 240-min treatment (Table 2). LPS did not affect the expression of ZFP36L1 gene but decreased mRNA levels of ZFP36L2 in cells treated for 120 and 240 min and those of ZFP36L3 gene in cells treated for 120 min (Table 2). Insulin did not affect the expression of the 3 TTP homologs in RAW cells (Table 2). ZFP36L1 protein was barely detected in RAW cells treated with the 3 agents (data not shown).

We also analyzed the expression of 2 additional classes of genes in RAW cells:. HuR/ELAVL1 (an mRNA-stabilizing protein with distinct but overlapping binding specificity with TTP) (30) and VEGF (a proangiogenic cytokine as a target of TTP family proteins) (31). CPE increased VEGFA mRNA levels only in cells treated for 240 min, but LPS and insulin did not affect VEGFA gene expression (Table 2). CPE, LPS, and insulin all decreased VEGFB mRNA levels in cells treated for 120 min, but LPS also decreased in those treated for 240 min (Table 2). CPE, LPS, and insulin did not affect the levels of mRNAs encoding HuR in RAW cells (data not shown).

CPE and LPS but not insulin increased TTP-targeted proinflammatory cytokine mRNA levels. TTP is a mRNA-destabilizing factor for a number of immediate-early response genes, such as TNF and GM-CSF genes (1). Therefore, selected proinflammatory cytokine gene expression was investigated in RAW264.7 macrophages after treatment with CPE, LPS, and insulin. TNF mRNA levels in RAW cells treated with 100 mg/L CPE for 30–240 min were 120–620% of those in the untreated controls (**Table 3**). Unlike CPE, insulin did not exhibit any effect on TNF mRNA levels in RAW cells (Table 3). LPS possessed a much stronger effect than CPE on TNF gene expression in RAW cells. TNF mRNA levels in cells treated with 0.1 mg/L LPS for 30–240 min were 11- to 49-fold of the controls (Table 3).

Both CPE and insulin did not affect GM-CSF mRNA levels in RAW macrophages (Table 3). LPS stimulation on GM-CSF gene expression was slow but with profound effects in RAW cells. GM-CSF mRNA levels in cells treated with 0.1 mg/L LPS for 60–240 min were 5- to 1260-fold of the controls (Table 3).

CPE increased COX2 and IL6 mRNA levels in RAW cells. CPE (100 mg/L) treatment for 60–240 min increased COX2 and IL6 mRNA levels by ~200–340% and 160–350% of the controls, respectively (Table 3). These increases were significant when the data from the control, CPE, and insulin treatments were analyzed separately (data not shown) but were not significant if the data from LPS treatment were included in the multiple comparison statistical analyses (Table 3). Insulin (100 nmol/L, 0.5–4 h) did not affect the expression of COX2 or IL6 gene (Table 3). COX2 and IL6 gene expression in RAW cells was stimulated by LPS more than by CPE. The mRNA levels of COX2 and IL6 in RAW cells treated with 0.1 mg/L LPS for 60–240 min

TABLE 2 Effects of CPE, insulin, and LPS on TTP family, VEGF, and HuR mRNA levels in mouse RAW264.7 macrophages[1,2]

mRNA	Time	Control	CPE	Insulin	LPS	*P*-value
	min			*Fold of control*		
TTP (ZFP36/TIS11)	30	1.08 ± 0.38[c]	1.59 ± 0.17[b]	1.21 ± 0.21[c]	9.10 ± 2.01[a]	0.007
	60	1.01 ± 0.07[c]	1.64 ± 0.16[b]	1.00 ± 0.04[c]	22.71 ± 4.63[a]	0.005
	120	1.00 ± 0.06[c]	1.88 ± 0.17[b]	0.89 ± 0.06[d]	10.57 ± 1.51[a]	0.003
	240	1.00 ± 0.08[c]	1.49 ± 0.15[b]	1.07 ± 0.14[c]	38.85 ± 4.34[a]	0.005
ZFP36L1 (TIS11B)	30	1.00 ± 0.06[a]	0.69 ± 0.05[b]	0.90 ± 0.11[a]	0.89 ± 0.03[a]	<0.001
	60	1.01 ± 0.12	0.98 ± 0.06	1.07 ± 0.17	1.14 ± 0.63	0.699
	120	1.01 ± 0.11	1.10 ± 0.14	1.07 ± 0.27	1.03 ± 0.16	0.873
	240	1.00 ± 0.07[a]	0.74 ± 0.13[b]	0.98 ± 0.05[a]	0.91 ± 0.05[a]	0.004
ZFP36L2 (TIS11D)	30	1.00 ± 0.06[a]	0.77 ± 0.12[b]	0.98 ± 0.08[a]	1.06 ± 0.07[a]	0.003
	60	1.01 ± 0.10	1.01 ± 0.12	1.02 ± 0.16	1.08 ± 0.32	0.879
	120	1.00 ± 0.12[a]	1.19 ± 0.10[a]	1.00 ± 0.13[a]	0.43 ± 0.05[b]	<0.001
	240	1.01 ± 0.12[a]	1.29 ± 0.24[a]	1.03 ± 0.11[a]	0.66 ± 0.10[b]	0.001
ZFP36L3	30	1.01 ± 0.15	0.84 ± 0.06	1.00 ± 0.18	0.87 ± 0.08	0.170
	60	1.00 ± 0.06	0.81 ± 0.07	1.08 ± 0.20	1.01 ± 0.39	0.058
	120	1.01 ± 0.14[a]	0.61 ± 0.04[b]	0.87 ± 0.26[a]	0.52 ± 0.06[b]	0.002
	240	1.01 ± 0.13[a]	0.68 ± 0.12[b]	0.93 ± 0.13[a]	0.87 ± 0.09[a]	0.012
VEGFA	30	1.03 ± 0.27	0.73 ± 0.08	0.87 ± 0.20	0.88 ± 0.13	0.235
	60	1.00 ± 0.11	0.98 ± 0.09	1.26 ± 0.23	1.31 ± 0.34	0.109
	120	1.01 ± 0.10	1.41 ± 0.38	1.05 ± 0.07	1.56 ± 0.48	0.153
	240	1.00 ± 0.12[b]	1.72 ± 0.21[a]	1.04 ± 0.25[b]	1.34 ± 0.36[ab]	0.006
VEGFB	30	1.01 ± 0.12	1.04 ± 0.07	1.05 ± 0.21	1.02 ± 0.04	0.966
	60	1.01 ± 0.18	1.13 ± 0.26	1.00 ± 0.15	1.13 ± 0.22	0.691
	120	1.00 ± 0.07[a]	0.72 ± 0.13[b]	0.78 ± 0.13[b]	0.60 ± 0.12[b]	0.003
	240	1.00 ± 0.12[a]	1.05 ± 0.12[a]	1.18 ± 0.19[a]	0.57 ± 0.01[b]	0.014

[1] Values are means ± SD, $n = 4$. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] RT-PCR method was identical to that described in the footnotes to Table 1. The relative ratios of mRNA levels were calculated using the $\Delta\Delta C_T$ method normalized with RPL32 C_T value as the internal control and the control C_T value as the calibrator. HuR (ELAVL1) mRNA levels did not differ among the treatments at any of the time points in RAW cells (data not shown).

were 24- to 532-fold and 24- to 1868-fold of those in the control cells, respectively (Table 3).

CPE and LPS but not insulin increased GLUT1 mRNA levels. Glucose is the major metabolic substrate critically important for host immunity (25,26). Glucose uptake in mammalian cells is facilitated by GLUT family proteins (27). Because none of the gene expression was apparently affected by insulin in the above PCR assays, we therefore analyzed the effects of insulin on the regulation of the GLUT family and related gene expression in RAW cells compared with those of CPE and LPS. Insulin did not affect the expression of GLUT family genes (**Table 4**). However, both CPE and LPS increased GLUT1 mRNA levels (the major GLUT mRNA in RAW cells; refer to Table 1) in RAW cells after 120 min treatment (Table 4). After 240 min treatment, CPE and LPS increased GLUT1 mRNA levels in RAW cells by more than 3- and 2-fold those of the control, respectively (Table 4). CPE did not affect the mRNA levels of GLUT3 (Table 4) but increased GLUT4 mRNA levels by >2-fold after 240 min treatment (Table 4). However, LPS decreased the mRNA levels of GLUT3 and GLUT4 (Table 4) in RAW cells.

INSR and GSK3B were selected to determine whether these 3 agents affected the components in the insulin signaling pathway. Insulin increased INSR mRNA levels in cells treated for 240 min (Table 4). CPE decreased INSR mRNA levels only in cells treated for 120 min, but LPS decreased those cells treated for 120 and

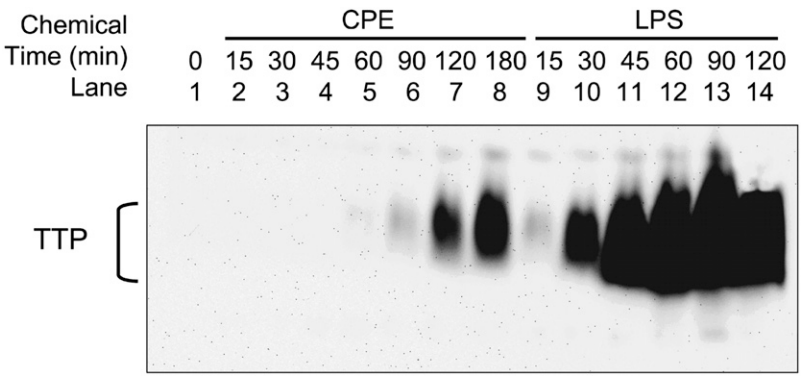

FIGURE 1 CPE and LPS but not insulin increased TTP protein levels in mouse RAW264.7 macrophages. Protein extracts were isolated from RAW cells treated with DMSO control (0.1%), insulin (100 nmol/L), CPE (100 mg/L), and LPS (0.1 mg/L) for various times. Proteins in the 10,000-g supernatants were separated by 10% SDS-PAGE. TTP was detected by immunoblotting with anti-MBP-TTP serum. Each lane was loaded with 100 μg of protein. Lane 1, DMSO control; lanes 2–8, CPE treatment; lanes 9–14, LPS treatment. Insulin did not affect TTP protein levels in the same cells (data not shown).

TABLE 3 Effects of CPE, insulin, and LPS on TNF, GM-CSF, COX2, IL6, and IFNγ mRNA levels in mouse RAW264.7 macrophages[1,2]

mRNA	Time	Control	CPE	Insulin	LPS	*P*-value
	min			*Fold of control*		
TNF	30	1.00 ± 0.09[b]	1.18 ± 0.13[b]	0.96 ± 0.11[b]	11.27 ± 2.77[a]	0.008
	60	1.01 ± 0.17[c]	1.80 ± 0.13[b]	1.00 ± 0.02[c]	12.18 ± 0.27[a]	0.010
	120	1.00 ± 0.09[c]	3.07 ± 0.20[b]	0.88 ± 0.11[c]	49.35 ± 4.44[a]	0.005
	240	1.02 ± 0.24[c]	6.18 ± 0.64[b]	1.01 ± 0.21[c]	47.75 ± 5.50[a]	0.005
GM-CSF (CSF2)	30	1.02 ± 0.23	0.60 ± 0.10	0.78 ± 0.27	1.01 ± 0.20	0.042
	60	1.04 ± 0.31[b]	1.27 ± 0.45[b]	1.40 ± 0.58[b]	4.97 ± 1.62[a]	0.028
	120	1.03 ± 0.27[b]	1.01 ± 0.23[b]	1.12 ± 0.39[b]	148.6 ± 21.4[a]	0.033
	240	1.03 ± 0.26[b]	1.11 ± 0.44[b]	0.88 ± 0.14[b]	1245.2 ± 83.6[a]	<0.001
COX2 (PTGS2)	30	1.04 ± 0.36[b]	1.07 ± 0.25[b]	0.70 ± 0.07[b]	2.25 ± 0.46[a]	0.028
	60	1.01 ± 0.12[b]	2.14 ± 0.39[b,3]	1.06 ± 0.07[b]	23.59 ± 1.85[a]	<0.001
	120	1.02 ± 0.25[b]	2.05 ± 0.18[b,3]	1.23 ± 0.34[b]	82.71 ± 0.81[a]	<0.001
	240	1.13 ± 0.27[b]	3.44 ± 1.08[b,3]	0.76 ± 0.06[b]	531.90 ± 7.82[a]	<0.001
IL6	30	1.18 ± 0.88	1.56 ± 0.24	1.43 ± 0.03	2.43 ± 0.07	0.172
	60	1.02 ± 0.28[b]	2.75 ± 0.52[b,3]	1.89 ± 0.20[b]	23.92 ± 0.94[a]	<0.001
	120	1.00 ± 0.10[b]	3.50 ± 1.37[b,3]	0.70 ± 0.04[b]	82.42 ± 9.30[a]	<0.001
	240	1.00 ± 0.14[b]	3.20 ± 0.44[b,3]	0.94 ± 0.65[b]	1865 ± 137.2[a]	<0.001

[1] Values are means ± SD, $n = 2$–4. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] RT-PCR method was identical to that described in the footnotes to Table 1. IFNγ mRNA levels were too low to be reliable for comparison (data not shown).
[3] Statistical analysis excluding LPS data indicates that the CPE COX2 and IL6 mRNA levels are significantly different from those of the control and insulin treatments. The results of all the other statistical analyses were the same with or without LPS data.

240 min (Table 4). None of the 3 agents significantly affected the mRNA levels of GSK3B gene (data not shown).

Discussion

TTP has antiinflammatory properties with therapeutic potential for the prevention and treatment of inflammation-related diseases. Previous studies have shown that TTP gene expression is induced in mammalian cells by a wide range of agents (1). However, most of the same agents also increase the expression levels of proinflammatory cytokines in the same cells and tissues (8). We have shown that TTP gene expression is induced by CPE in mouse 3T3-L1 adipocytes (17). In this study, we determined that CPE also exhibited the ability to rapidly increase TTP mRNA and protein in mouse RAW264.7 macrophages. However, CPE also increased the levels of mRNAs coding for proinflammatory cytokines, including TNF, COX2, and IL6 in the same cells, but to much less extent than LPS and with a slower response than TTP.

TABLE 4 Effects of CPE, insulin, and LPS on GLUT family, INSR, and GSK3B mRNA levels in mouse RAW264.7 macrophages[1,2]

mRNA	Time	Control	CPE	Insulin	LPS	*P*-value
	min			*Fold of control*		
GLUT1 (SLC2A1)	30	1.01 ± 0.20	0.83 ± 0.02	0.84 ± 0.19	0.82 ± 0.09	0.178
	60	1.00 ± 0.08[b]	1.12 ± 0.17[b]	1.07 ± 0.14[b]	1.43 ± 0.25[a]	0.008
	120	1.00 ± 0.08[c]	1.60 ± 0.35[a]	1.11 ± 0.09[b]	1.92 ± 0.43[a]	<0.001
	240	1.00 ± 0.18[c]	3.17 ± 0.20[a]	1.18 ± 0.21[c]	2.33 ± 0.36[b]	<0.001
GLUT3 (SLC2A3)	30	1.01 ± 0.12	0.92 ± 0.07	0.96 ± 0.13	0.88 ± 0.09	0.196
	60	1.02 ± 0.19	1.10 ± 0.13	1.12 ± 0.07	0.92 ± 0.16	0.099
	120	1.01 ± 0.17[a]	1.06 ± 0.23[a]	0.94 ± 0.13[a]	0.65 ± 0.15[b]	0.003
	240	1.01 ± 0.11[a]	1.14 ± 0.09[a]	1.08 ± 0.13[a]	0.70 ± 0.10[b]	<0.001
GLUT4 (SLC2A4)	30	1.02 ± 0.24	0.87 ± 0.37	0.80 ± 0.13	0.81 ± 0.08	0.354
	60	1.03 ± 0.32	0.83 ± 0.07	0.98 ± 0.21	0.77 ± 0.15	0.066
	120	1.02 ± 0.21[b]	1.34 ± 0.23[a]	0.91 ± 0.22[b]	0.28 ± 0.07[c]	<0.001
	240	1.02 ± 0.19[b]	2.37 ± 0.32[a]	1.18 ± 0.12[b]	0.31 ± 0.07[c]	<0.001
INSR	30	1.00 ± 0.05	0.83 ± 0.15	0.91 ± 0.11	0.93 ± 0.06	0.178
	60	1.01 ± 0.18	0.93 ± 0.07	1.06 ± 0.13	1.05 ± 0.22	0.656
	120	1.01 ± 0.09[a]	0.69 ± 0.12[b]	0.90 ± 0.03[a]	0.68 ± 0.15[b]	0.002
	240	1.01 ± 0.09[b]	1.03 ± 0.08[b]	1.23 ± 0.16[a]	0.61 ± 0.04[c]	<0.001

[1] Values are means ± SD, $n = 4$–6. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] RT-PCR method was identical to that described in the footnotes to Table 1. GLUT2 mRNA was undetectable under the PCR assay conditions (data not shown). GSK3B mRNA levels were not significantly different among the treatments in any of the time points in RAW cells (data not shown).

One major finding of this study is that CPE increased TTP mRNA and protein levels in mouse RAW macrophages. TTP is a highly phosphorylated protein (32,33) with antiinflammatory functions through down-regulation of proinflammatory cytokines (1). TTP binds to AU-rich elements in some mRNA sequences and destabilizes those transcripts encoding proteins such as TNFα (6–9), GM-CSF (10), and COX2 (12). The size of TTP was similar in CPE- and LPS-stimulated TTP in RAW cells (Fig. 1) (19), suggesting that TTP might be phosphorylated in CPE-treated RAW cells. CPE increased TTP mRNA levels after 30 min treatment and TTP protein was clearly detected after 90 min treatment in RAW cells. The current finding that CPE increased TTP gene expression in macrophages is consistent with previous findings that TTP gene expression is increased by CPE in adipocytes (17). These results suggest that nutritional supplements with cinnamon may therefore affect modulating immunological responses in mammalian species.

The relative ratios of anti- and proinflammatory proteins are proposed to be important in the understanding of modulation of inflammatory responses (34). The 2-fold increases in TTP expression by CPE in RAW macrophages were sustained during the 4 h of treatment. However, the expression of a number of proinflammatory cytokine genes was gradually increased in RAW cells by CPE after treatment for longer times. Because TTP mRNA levels in unstimulated RAW cells were 6- to 3000-fold of TNF, COX2, GM-CSF, and IL6 mRNA levels (Table 1), the net increases of TTP mRNA molecules were still larger than those of the proinflammatory cytokine mRNA molecules in the same cells (**Supplemental Table 2**). Therefore, CPE may have benefits in improving inflammation-related conditions. However, additional studies are required to draw a firm conclusion, because the relationships between TTP mRNA levels, protein levels, and the interactions between TTP protein and its proinflammatory cytokine mRNA target molecules are not completely understood.

Another major finding in this study is that CPE and LPS, but not insulin, increased the levels of GLUT1 mRNA, the major form of GLUT family mRNAs in RAW macrophages. These results suggest that CPE and LPS may have profound effects on the regulation of energy metabolism in immunity-related macrophages, because glucose is the major metabolic substrate critically important for the host response to injury and infection (25,26). Our PCR results showed that GLUT1 mRNA levels were ~8-fold of GLUT3 and 1300-fold of GLUT4 mRNA levels and GLUT2 mRNA was undetectable in RAW cells. Previous studies reported that GLUT1 was the major if not the only GLUT in murine peritoneal macrophages that is induced by LPS, TNFα, burn injury, and *Pseudomonas aeruginosa* infection (25). Furthermore, increased GLUT1 gene expression results in enhanced glucose uptake in these macrophages (25,27). Our ability to detect GLUT3 and GLUT4 mRNAs in RAW cells is probably due to the more sensitive real-time PCR method we used. This pattern of GLUT family gene expression in RAW macrophages is different from those in rat liver and skeletal muscle, in which GLUT2 and GLUT4 are the major GLUT mRNAs in respective tissues (35). The ineffectiveness of insulin on GLUT1 gene expression in RAW cells is in agreement with a previous report that showed by flow cytometry that GLUT1 protein does not respond to insulin in either resting or phorbol 12-myristate 13-acetate/LPS-activated white blood cells from healthy people (36).

The results reported here demonstrate that CPE, unlike insulin, is able to promote TTP, GLUT1, and proinflammatory gene expression in RAW macrophages. Our results suggest that RAW macrophages are probably insensitive to insulin, because none of the mRNA levels evaluated were significantly affected by insulin treatment, except that insulin slightly decreased TTP and VEGFB mRNA levels in cells treated for 120 min and increased INSR mRNA levels in cells treated for 240 min. These results are in agreement with the noninsulin-dependent glucose uptake in macrophage-rich tissues, such as liver, spleen, and lung (37), which is opposite to the effect of insulin on GLUT4 gene expression in 3T3-L1 adipocytes (38). Previous studies suggest that cinnamon exhibits insulin-like activity in cells, animals, and people with type 2 diabetes. Both cinnamon and insulin increase antiinflammatory TTP gene expression in mouse adipocytes (17,28), increase the activity of autophosphorylation of the INSRβ and decrease the activity of tyrosine phosphatase in vitro (39), and stimulate glucose uptake and glycogen biosynthesis, activate glycogen synthase, and inhibit glycogen synthase kinase-3β (40). CPE potentiates in vivo insulin-regulated glucose utilization via increasing glucose uptake (41). It also decreases glucose and increases insulin in blood of rats (42) and decreases blood pressure (43). Cinnamon powder also decreased the levels of glucose, triglycerides, and LDL cholesterol in people with type 2 diabetes (44) and delayed gastric emptying without affecting satiety (45). Cinnamon and its polyphenol extract exhibit both insulin-dependent effects in insulin-sensitive cells like adipocytes and insulin-independent effects in insulin-insensitive cells like macrophages. Therefore, the benefit of cinnamon is likely due to its multiple effects, including insulin potentiation and antiinflammatory effects.

The major differences in gene expression affected by CPE and LPS were that: *1*) CPE had a greater effect on GLUT1 gene expression than LPS, but LPS had much greater effects than CPE in TTP and proinflammatory cytokine gene expression; *2*) CPE increased only TTP gene expression, but LPS also induced TTP, TNF, and COX2 gene expression in RAW cells treated for 30 min; *3*) GM-CSF mRNA levels were induced only by LPS by not by CPE in RAW cells; and *4*) CPE did not affect GLUT3 gene expression and increased GLUT4 gene expression, but LPS decreased both in RAW cells. These differences may explain the observed beneficial effects of CPE as nutrient supplement and the detrimental effects of LPS. The sustained TTP gene expression patterns stimulated by CPE and LPS in RAW macrophages are similar to those by CPE in 3T3-L1 adipocytes (17), by LPS in RAW264.7 cells (19), and by glucocorticoids in A549 lung epithelial cells and rat tissues (46) but are different from the transient induction by insulin in HIR3.5 preadipocytes (3) and 3T3-L1 adipocytes (28).

In summary, this study reports several novel findings: *1*) CPE rapidly induces TTP mRNA and protein and induces TTP gene expression more rapidly than the proinflammatory cytokine mRNAs encoding TNFα, COX2, and IL6 in mouse macrophages; *2*) the net increase in TTP mRNA levels was greater than the proinflammatory cytokines measured; *3*) CPE increases more GLUT1 gene expression than LPS; *4*) CPE effects on gene expression patterns are different from those of LPS in RAW macrophages during the initial treatment; and *5*) insulin does not exhibit major effects on the expression of these genes. Our results indicate that CPE is capable of affecting immune responses by regulating anti- and proinflammatory as well as the gene expression of GLUT in macrophages.

Acknowledgments

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Literature Cited

1. Blackshear PJ. Tristetraprolin and other CCCH tandem zinc-finger proteins in the regulation of mRNA turnover. Biochem Soc Trans. 2002; 30:945–52.

2. Blackshear PJ, Phillips RS, Ghosh S, Ramos SB, Richfield EK, Lai WS. Zfp36l3, a rodent X chromosome gene encoding a placenta-specific member of the tristetraprolin family of CCCH tandem zinc finger proteins. Biol Reprod. 2005;73:297–307.
3. Lai WS, Stumpo DJ, Blackshear PJ. Rapid insulin-stimulated accumulation of an mRNA encoding a proline-rich protein. J Biol Chem. 1990;265:16556–63.
4. DuBois RN, McLane MW, Ryder K, Lau LF, Nathans D. A growth factor-inducible nuclear protein with a novel cysteine/histidine repetitive sequence. J Biol Chem. 1990;265:19185–91.
5. Varnum BC, Lim RW, Kujubu DA, Luner SJ, Kaufman SE, Greenberger JS, Gasson JC, Herschman HR. Granulocyte-macrophage colony-stimulating factor and tetradecanoyl phorbol acetate induce a distinct, restricted subset of primary-response TIS genes in both proliferating and terminally differentiated myeloid cells. Mol Cell Biol. 1989;9:3580–3.
6. Cao H, Dzineku F, Blackshear PJ. Expression and purification of recombinant tristetraprolin that can bind to tumor necrosis factor-alpha mRNA and serve as a substrate for mitogen-activated protein kinases. Arch Biochem Biophys. 2003;412:106–20.
7. Cao H. Expression, purification, and biochemical characterization of the antiinflammatory tristetraprolin: a zinc-dependent mRNA binding protein affected by posttranslational modifications. Biochemistry. 2004;43:13724–38.
8. Carballo E, Lai WS, Blackshear PJ. Feedback inhibition of macrophage tumor necrosis factor-alpha production by tristetraprolin. Science. 1998;281:1001–5.
9. Lai WS, Carballo E, Strum JR, Kennington EA, Phillips RS, Blackshear PJ. Evidence that tristetraprolin binds to AU-rich elements and promotes the deadenylation and destabilization of tumor necrosis factor alpha mRNA. Mol Cell Biol. 1999;19:4311–23.
10. Carballo E, Lai WS, Blackshear PJ. Evidence that tristetraprolin is a physiological regulator of granulocyte-macrophage colony-stimulating factor messenger RNA deadenylation and stability. Blood. 2000;95:1891–9.
11. Carballo E, Cao H, Lai WS, Kennington EA, Campbell D, Blackshear PJ. Decreased sensitivity of tristetraprolin-deficient cells to p38 inhibitors suggests the involvement of tristetraprolin in the p38 signaling pathway. J Biol Chem. 2001;276:42580–7.
12. Sawaoka H, Dixon DA, Oates JA, Boutaud O. Tristetraprolin binds to the 3′-untranslated region of cyclooxygenase-2 mRNA. A polyadenylation variant in a cancer cell line lacks the binding site. J Biol Chem. 2003;278:13928–35.
13. Phillips K, Kedersha N, Shen L, Blackshear PJ, Anderson P. Arthritis suppressor genes TIA-1 and TTP dampen the expression of tumor necrosis factor alpha, cyclooxygenase 2, and inflammatory arthritis. Proc Natl Acad Sci USA. 2004;101:2011–6.
14. Taylor GA, Carballo E, Lee DM, Lai WS, Thompson MJ, Patel DD, Schenkman DI, Gilkeson GS, Broxmeyer HE, et al. A pathogenetic role for TNF alpha in the syndrome of cachexia, arthritis, and autoimmunity resulting from tristetraprolin (TTP) deficiency. Immunity. 1996;4:445–54.
15. Sauer I, Schaljo B, Vogl C, Gattermeier I, Kolbe T, Muller M, Blackshear PJ, Kovarik P. Interferons limit inflammatory responses by induction of tristetraprolin. Blood. 2006;107:4790–7.
16. Cousins RJ, Blanchard RK, Popp MP, Liu L, Cao J, Moore JB, Green CL. A global view of the selectivity of zinc deprivation and excess on genes expressed in human THP-1 mononuclear cells. Proc Natl Acad Sci USA. 2003;100:6952–7.
17. Cao H, Polansky MM, Anderson RA. Cinnamon extract and polyphenols affect the expression of tristetraprolin, insulin receptor, and glucose transporter 4 in mouse 3T3–L1 adipocytes. Arch Biochem Biophys. 2007;459:214–22.
18. Cao H, Kelly MA, Kari F, Dawson HD, Urban JF Jr, Coves S, Roussel AM, Anderson RA. Green tea increases anti-inflammatory tristetraprolin and decreases pro-inflammatory tumor necrosis factor mRNA levels in rats. J Inflamm (Lond). 2007;4:1.
19. Cao H, Tuttle JS, Blackshear PJ. Immunological characterization of tristetraprolin as a low abundance, inducible, stable cytosolic protein. J Biol Chem. 2004;279:21489–99.
20. Taddeo B, Zhang W, Roizman B. The U(L)41 protein of herpes simplex virus 1 degrades RNA by endonucleolytic cleavage in absence of other cellular or viral proteins. Proc Natl Acad Sci USA. 2006;103:2827–32.
21. Broadhurst CL, Polansky MM, Anderson RA. Insulin-like biological activity of culinary and medicinal plant aqueous extracts in vitro. J Agric Food Chem. 2000;48:849–52.
22. Anderson RA, Broadhurst CL, Polansky MM, Schmidt WF, Khan A, Flanagan VP, Schoene NW, Graves DJ. Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. J Agric Food Chem. 2004;52:65–70.
23. Shan B, Cai YZ, Brooks JD, Corke H. Antibacterial properties and major bioactive components of cinnamon stick (Cinnamomum burmannii): activity against foodborne pathogenic bacteria. J Agric Food Chem. 2007;55:5484–90.
24. Zhu W, Brauchle MA, Di Padova F, Gram H, New L, Ono K, Downey JS, Han J. Gene suppression by tristetraprolin and release by the p38 pathway. Am J Physiol Lung Cell Mol Physiol. 2001;281:L499–508.
25. Gamelli RL, Liu H, He LK, Hofmann CA. Augmentations of glucose uptake and glucose transporter-1 in macrophages following thermal injury and sepsis in mice. J Leukoc Biol. 1996;59:639–47.
26. Calder PC, Dimitriadis G, Newsholme P. Glucose metabolism in lymphoid and inflammatory cells and tissues. Curr Opin Clin Nutr Metab Care. 2007;10:531–40.
27. Fukuzumi M, Shinomiya H, Shimizu Y, Ohishi K, Utsumi S. Endotoxin-induced enhancement of glucose influx into murine peritoneal macrophages via GLUT1. Infect Immun. 1996;64:108–12.
28. Cao H, Urban JF Jr, Anderson RA. Insulin increases tristetraprolin and decreases VEGF gene expression in mouse 3T3–L1 adipocytes. Obesity (Silver Spring). 16: In press 2008.
29. Cao H, Lin R, Ghosh S, Anderson RA, Urban JF Jr. Production and characterization of ZFP36L1 antiserum against recombinant protein from *Escherichia coli*. Biotechnol Prog. 2008;24:326–33.
30. Raghavan A, Robison RL, McNabb J, Miller CR, Williams DA, Bohjanen PR. HuA and tristetraprolin are induced following T cell activation and display distinct but overlapping RNA binding specificities. J Biol Chem. 2001;276:47958–65.
31. Essafi-Benkhadir K, Onesto C, Stebe E, Moroni C, Pages G. Tristetraprolin inhibits ras-dependent tumor vascularization by inducing VEGF mRNA degradation. Mol Biol Cell. 2007;18:4648–58.
32. Cao H, Deterding LJ, Venable JD, Kennington EA, Yates JR III, Tomer KB, Blackshear PJ. Identification of the anti-inflammatory protein tristetraprolin as a hyperphosphorylated protein by mass spectrometry and site-directed mutagenesis. Biochem J. 2006;394:285–97.
33. Cao H, Deterding LJ, Blackshear PJ. Phosphorylation site analysis of the anti-inflammatory and mRNA-destabilizing protein tristetraprolin. Expert Rev Proteomics. 2007;4:711–26.
34. Frasca D, Landin AM, Alvarez JP, Blackshear PJ, Riley RL, Blomberg BB. Tristetraprolin, a negative regulator of mRNA stability, is increased in old B cells and is involved in the degradation of e47 mRNA. J Immunol. 2007;179:918–27.
35. Cao H, Hininger-Favier I, Kelly MA, Benaraba R, Dawson HD, Coves S, Roussel AM, Anderson RA. Green tea polyphenol extract regulates the expression of genes involved in glucose uptake and insulin signaling in rats fed a high fructose diet. J Agric Food Chem. 2007;55:6372–8.
36. Maratou E, Dimitriadis G, Kollias A, Boutati E, Lambadiari V, Mitrou P, Raptis SA. Glucose transporter expression on the plasma membrane of resting and activated white blood cells. Eur J Clin Invest. 2007;37:282–90.
37. Meszaros K, Lang CH, Bagby GJ, Spitzer JJ. In vivo glucose utilization by individual tissues during nonlethal hypermetabolic sepsis. FASEB J. 1988;2:3083–6.
38. Taha C, Liu Z, Jin J, Al-Hasani H, Sonenberg N, Klip A. Opposite translational control of GLUT1 and GLUT4 glucose transporter mRNAs in response to insulin. Role of mammalian target of rapamycin, protein kinase b, and phosphatidylinositol 3-kinase in GLUT1 mRNA translation. J Biol Chem. 1999;274:33085–91.
39. Imparl-Radosevich J, Deas S, Polansky MM, Baedke DA, Ingebritsen TS, Anderson RA, Graves DJ. Regulation of PTP-1 and insulin receptor kinase by fractions from cinnamon: implications for cinnamon regulation of insulin signalling. Horm Res. 1998;50:177–82.
40. Jarvill-Taylor KJ, Anderson RA, Graves DJ. A hydroxychalcone derived from cinnamon functions as a mimetic for insulin in 3T3–L1 adipocytes. J Am Coll Nutr. 2001;20:327–36.
41. Qin B, Nagasaki M, Ren M, Bajotto G, Oshida Y, Sato Y. Cinnamon extract (traditional herb) potentiates in vivo insulin-regulated glucose

utilization via enhancing insulin signaling in rats. Diabetes Res Clin Pract. 2003;62:139–48.
42. Verspohl EJ, Bauer K, Neddermann E. Antidiabetic effect of Cinnamomum cassia and Cinnamomum zeylanicum in vivo and in vitro. Phytother Res. 2005;19:203–6.
43. Preuss HG, Echard B, Polansky MM, Anderson R. Whole cinnamon and aqueous extracts ameliorate sucrose-induced blood pressure elevations in spontaneously hypertensive rats. J Am Coll Nutr. 2006;25:144–50.
44. Khan A, Safdar M, li Khan MM, Khattak KN, Anderson RA. Cinnamon improves glucose and lipids of people with type 2 diabetes. Diabetes Care. 2003;26:3215–8.
45. Hlebowicz J, Darwiche G, Bjorgell O, Almer LO. Effect of cinnamon on postprandial blood glucose, gastric emptying, and satiety in healthy subjects. Am J Clin Nutr. 2007;85:1552–6.
46. Smoak K, Cidlowski JA. Glucocorticoids regulate tristetraprolin synthesis and posttranscriptionally regulate tumor necrosis factor alpha inflammatory signaling. Mol Cell Biol. 2006;26:9126–35.